

The ultrastructure of palmyrah (*Borassus flabellifer* L) - intoxicated mouse hepatocytes

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Abstract

Adult male BALB/cJ mice fed with 40% palmyrah (*Borassus flabellifer* L) flour were examined at intervals of 1-5 days. Their livers were examined by light and electron microscopy. The hepatocytes of flour fed mice showed profound changes compared with the liver cells of control mice fed pellets alone. On light microscopy, there was hydropic degeneration predominantly of perivenular hepatocytes, and mild periportal fatty degeneration without necrosis. Ultrastructural changes consisted of progressive mitochondrial swelling with loss of cristae, vesiculation of rough endoplasmic reticulum, with disaggregation and degranulation of ribosomes. These observations confirm the biochemical evidence of the occurrence of palmyrah-induced mitochondrial damage demonstrated by earlier workers. This report describes ultrastructural changes in the liver induced by palmyrah flour.

Key words: palmyrah toxicity, mouse hepatocytes, ultrastructure.

Introduction

The flour derived from the young subterranean shoot of the palmyrah palm (*Borassus flabellifer* L) is used as a human food in some Asian and African countries. This flour has been shown to possess a wide range of toxic effects in acute and chronic feeding experiments in rats and mice (1, 2). With oral dosing of the flour, the histopathological changes were predominantly in the liver in both short term-high dose (severe centrilobular congestion, sinusoidal dilatation, hydropic degeneration and periportal fatty change) and in long term-low dose feeding (portal and central venous occlusion) in experimental rats.

This paper describes the ultrastructure of the mouse hepatocytes after short-term medium-dose oral feeding of palmyrah flour and attempts to correlate the light microscopic and biochemical findings described in earlier reports, with the ultrastructural lesions found in the present study.

Material and Methods

Animals

A total of 40 adult, male BALB/cJ in-bred mice, 6-8 weeks old, were used in 4 experiments.

Flour

The flour was obtained by grinding freshly harvested, boiled, sun-dried palmyrah shoots as described previously (1). The powder was stored at 4° C and the feed was replenished daily with fresh stocks to avoid fungal and mycotoxin contamination, with prolonged exposure.

Feeding

Three schedules of feeding were adopted with tap water *ad libitum* with each schedule; (a) Control mice were fed a normal diet of powdered pellets alone (controls) and (b) test mice were fed flour mixed with powdered pellets (25% or 40%) or (3) flour alone (100%). Each mouse received 2.6 gm of the fresh diet (pellet powder or flour-pellet mixture) each day. The flour had a caloric value of 1.72 mJ/100 gm as compared with 1.49 mJ/100 gm of the pellets.

Necropsy and light microscopy

At selected intervals of time, the mice were killed by cervical dislocation and slices of the liver were

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fixed in 10% buffered formalin, embedded in paraffin, sectioned at 4 μm and stained with haematoxylin-eosin. In addition, where necessary, stains for reticulin (Gordon & Sweet) and intracytoplasmic glycogen (periodic acid-Schiff reaction, PAS) were also used.

Electron microscopy

Parallel slices of liver 1 mm^3 were fixed in 4% glutaraldehyde in phosphate buffer (pH 7.4), and post-fixed in 1% osmium tetroxide in cacodylate buffer. The tissue was dehydrated at room temperature and embedded in Epon 812. 1 μm thick sections were prepared and stained with 1% toluidine blue. Ultrathin sections (40-60 nm) were stained with uranyl acetate and lead citrate. The sections were viewed through a Hitachi HS-8 electron microscope at 50 Kv accelerating voltage.

Results

Light microscopy

Light microscopic changes were apparent in the flour-fed mice only after the third day. Lesions were most consistently demonstrated in mice fed 40% flour; 100% flour proved too toxic and unpalatable to many mice, leading to decreased or irregular intake after the second day and results were not reproducible.

By day 3, hepatocytes showed centrilobular congestion with sinusoidal dilatation and perivenous hepatocellular hydropic degeneration. The affected hepatocytes were enlarged, with fine granularity and clumping of cytoplasm.

With prolonged feeding of 40% flour, by days 3-5, the histological lesions increased in severity. Periportal fatty degeneration was noted, initially occurring focally, and later spreading to involve the majority of liver lobules. Hepatocellular necrosis was singularly absent.

The PAS reaction demonstrated a consistent reduction in the glycogen content of centrilobular hepatocytes. Reticulin collapse was not observed. Palmyrah flour appears to cause liver injury without hepatocellular necrosis, in the mouse. The

observations noted in this study are similar to those recorded in earlier studies of palmyrah hepatotoxicity in rats: the latter studies (histological, histochemical and biochemical) suggested mitochondrial injury as an early and well marked (if not primary) event in *in vivo*, *in vitro/in vivo* and in *in vitro* experiments (1).

Ultramicroscopic changes

Normal mice

The ultrastructure of the hepatocyte in a normal mouse at day 5 of feeding of rat pellets is shown in Fig. 1.

Palmyrah-fed mice

Alterations in the mitochondria and rough endoplasmic reticulum were the earliest detected changes, evident at 24 hr.

Mitochondria

There was increased lucency of the mitochondrial matrix, with progressive decrease in matricial density as the lesions progressed in severity (Fig. 2); swollen mitochondria contained increased numbers of intra-matrical dense deposits in the early stages of intoxication, but the numbers subsequently decreased. There was progressively severe loss of mitochondrial cristae as the lesions evolved (Fig. 3). Increasing severity of hydropic degeneration noted on light microscopy was paralleled by an equally severe mitochondrial hydrops (Fig. 3). By the 5th day, frank mitochondrial cavitation was noted, with rupture of mitochondrial membranes, and herniation of mitochondrial contents into the cytoplasm (Fig. 4). An increase in the number of microbodies was also noted.

Endoplasmic reticulum

Dilatation of the rough endoplasmic reticulum (RER) with increase in smooth endoplasmic reticulum (SER) (Fig. 5), degranulation and disaggregation of the ribosomes with an increase in free ribosomes in the cytoplasm (Fig. 6) were noted as early as 24 hr after feeding commenced.

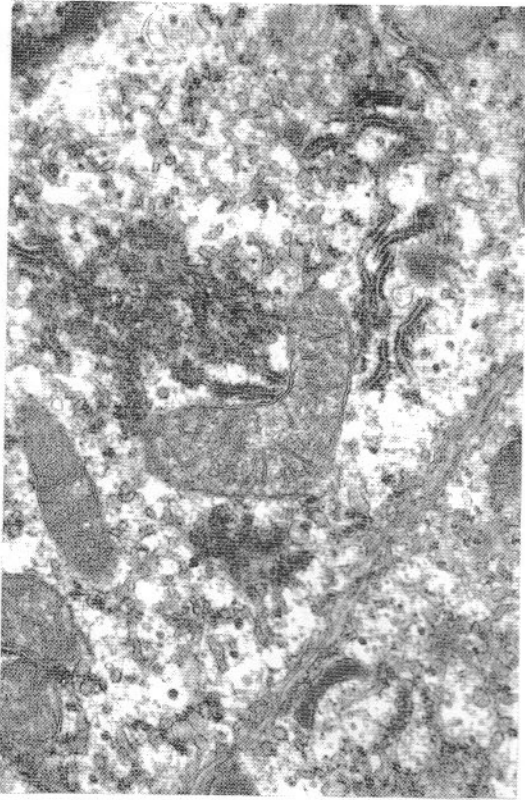


Figure 1: Normal control mouse liver. Fifth day of rat pellet feeding. x 19,000.

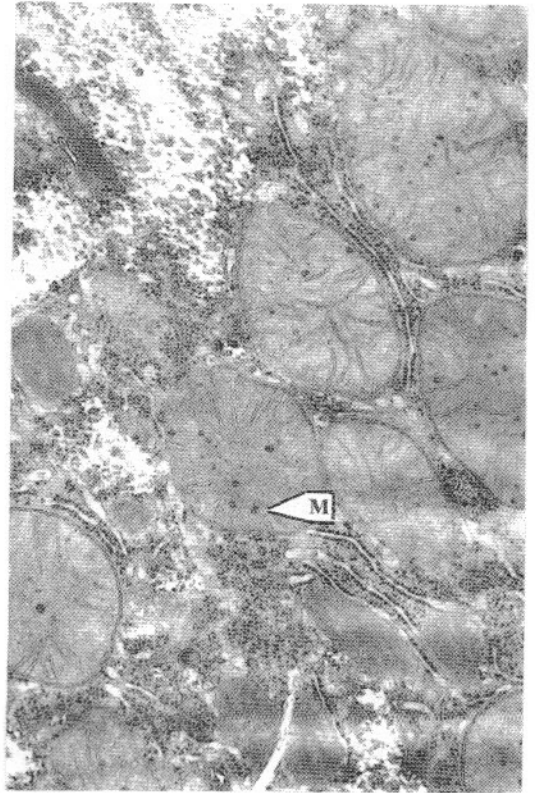


Figure 2: Mouse liver at third day of palmyrah feeding showing mitochondrial swelling, increased lucency of matrix and presence of micro-bodies (M). x 24,000.

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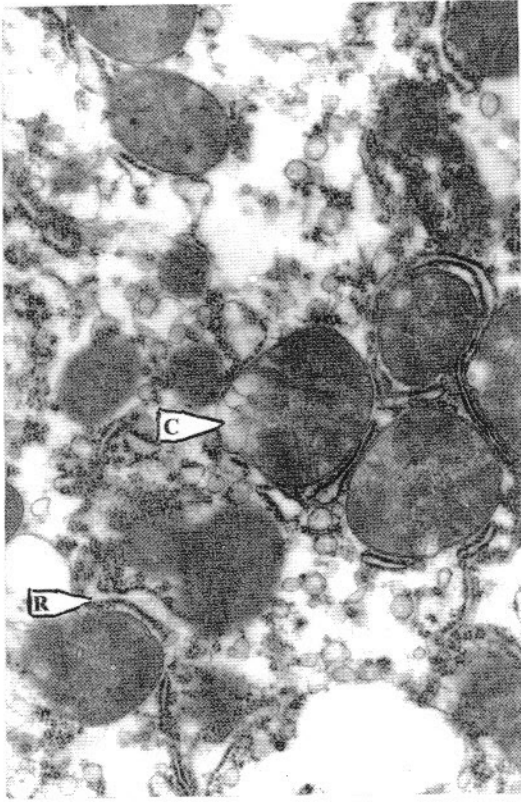


Figure 3: Fifth day of palmyrah feeding. Dilatation of RER (R), cavitation of mitochondria (C) with loss of cristae, and increase in SER. x 24,000.

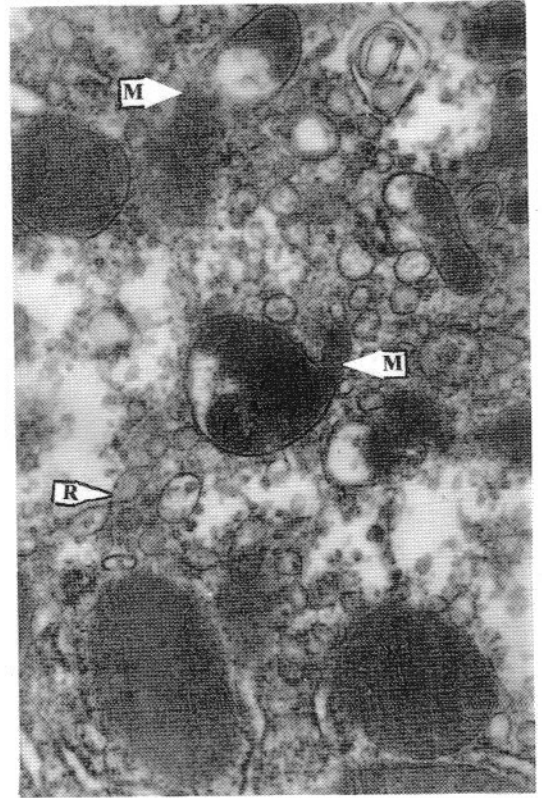


Figure 4: Fifth day of palmyrah feeding. Dilatation of RER (R), mitochondrial swelling with cavitation and herniation of contents through abnormal membrane (M); micro-body formation is less marked. x 24,000.

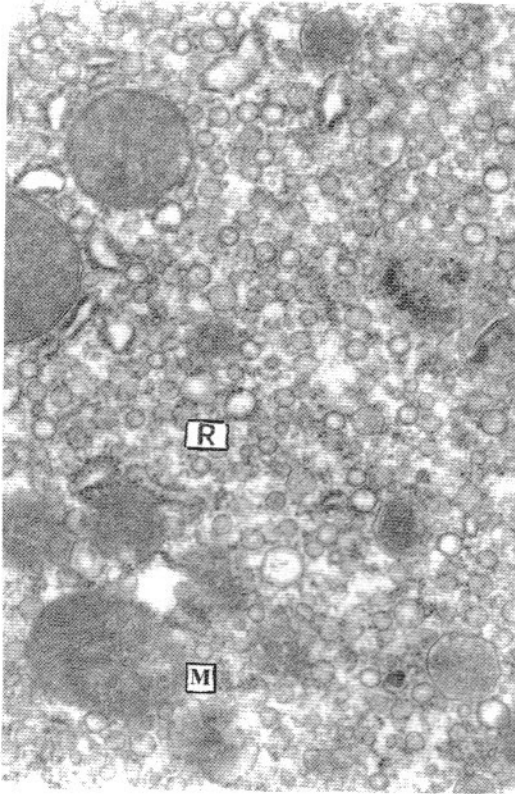


Figure 5: Third day of palmyrah feeding. Marked dilatation of RER (R) and hyperplasia of SER. Mitochondria show loss of cristae and abnormalities of membrane (M). x 24,000.

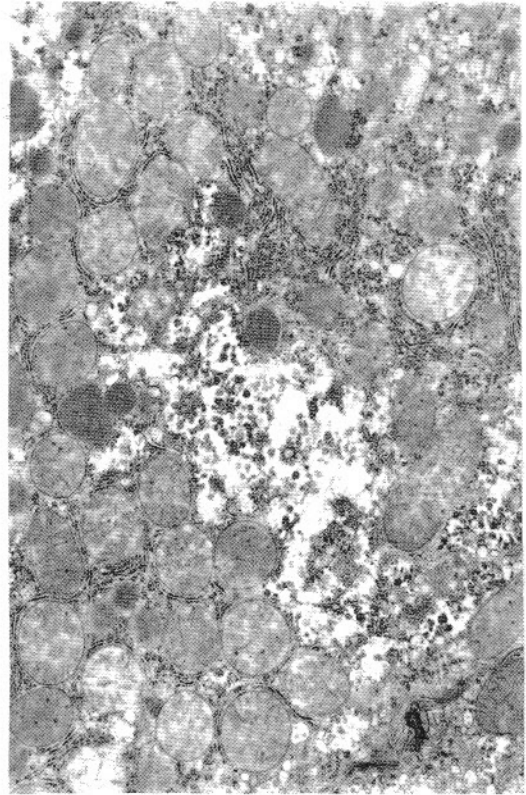


Figure 6: Fifth day of palmyrah feeding. Dilatation of RER and disaggregation of ribosomes. Although degranulation of ribosomes is also seen, many single ribosomes remain attached to the rough endoplasmic membrane. Clusters of free ribosomes are also present. x 24,000.

Discussion

The present findings confirm the occurrence of mitochondrial damage. Although it may be entirely possible that these changes may be simply a secondary manifestation of general metabolic deterioration in a damaged cell, the time-sequence analysis of the mitochondrial and RER changes noted in this study suggest that they are causally important in the pathology of the hepatocyte.

The isolation and identification of the hepatotoxic agent(s) in palmyrah flour has not yet been achieved although it has been shown that the partially purified immunosuppressive factors(s) do not seem to be associated with hepatotoxicity (Pathmanathan, unpublished observations, 1985). The palmyrah-induced lesions bear a similarity to those observed in the rat liver in ethionine toxicity, both at the light and ultramicroscopical levels (3, 4, 5, 6). It is currently believed that most of the hepatotoxic effects in acute ethionine poisoning are a result of low levels of cellular ATP. Judah *et al.*, (6) demonstrated a gross depletion of intracellular ATP leading to an increase in Na⁺ and a diminution of K⁺ ions intracellularly. This paralysis of an active mechanism for secretion and transport of protein molecules into the Golgi complex may be contributory to the changes in the RER. Biochemical studies (P.A.J. Perera and S.N. Arseculeratne, unpublished observations, 1983) on palmyrah intoxicated rats showed a significant depression of serum levels of alkaline phosphatase and alanine aminotransferase within 4-7 days of feeding. The hepatic cytosol showed normal levels of these enzymes. Cortisone and tryptophane induction of hepatic tryptophan oxygenase was normal, indicating perhaps a defect in the cell-to-serum transport of the alkaline phosphatase and alanine aminotransferase rather than one of disordered protein synthesis. C-14 labelled leucine incorporation in the hepatocyte

cytosol upto 5 days of palmyrah-feeding was normal, again suggesting that protein synthesis was undisturbed and unlikely to be the cause of the depression of serum levels of these enzymes. In this study, however, we were unable to demonstrate ultrastructural evidence of damage to the hepatic cell membrane, in palmyrah fed mice which might have explained the lowering of serum levels of hepatic enzymes.

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